

THE CHEMISTRY OF THE BARK
OF
ROBINIA PSEUD-ACACIA, LINNÉ

[False Acacia or Common Locust.]

BY
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Horse Ate Locust Bark.

Kilmer, of Schoharie, lost one
valuable horses recently. The
had been driven to Middleburgh
while there was hitched to a
tree in the yard of George B.
The horse died as the result
eating the bark of the tree. The
of a locust tree is poisonous to
as well as animals.

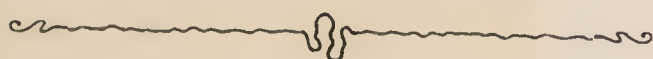
*The Antwerp
Republican
Schoharie, N. Y.
Jan 28. 1908*

ground hog comes out of
place to look around, and if
happen to see his shadow
casion Platt V. Washburn
ready right away to cut so

Standard Rain and Snow
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The Chemistry of the Bark of Robinia Pseud-acacia, Linné.*

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In a paper read by me before the Wisconsin Academy of Sciences, Arts and Letters, on December 27, 1889, and published in the *Pharm. Rundschau*, New York, February, 1890, pp. 29-38, entitled: "On the Chemical Constituents and Poisonous Principle of the Bark of *Robinia Pseud-acacia*, Linné" (Power and Cambier), an account was given of some recent severe cases of poisoning produced by chewing the bark of the Robinia, together with an investigation which had been undertaken during the preceding summer for the purpose of determining the nature of the poisonous principle. In the paper referred to (abstracted in the *Pharm. Journ.*, 1890, p. 711), the more commonly occurring constituents of the bark were noted, as also the isolation of a substance which was precipitated by all the usual alkaloidal reagents and believed to be choline, although observed to differ from the latter in some of its characters. The most important observation, however, to which attention was directed was the fact that the toxic action of Robinia bark is due to a poisonous proteid, similar in its character to ricin and abrin, and, being guided by the classification of the albuminoids adopted at that time by

* Read before the British Pharmaceutical Conference, Dublin, July, 1901, and reprinted from the *Pharmaceutical Journal*, August 17 and 24, 1901.

Hoppe-Seyler, it was stated that "it would appear to be correctly and conveniently designated as a *phyt-albumose*."

In the former publication some of the general chemical characters of this proteid were described, and its extremely poisonous action—manifested by severe and prolonged attacks of vomiting and purging—was also fully and satisfactorily established by experiments upon myself and by my assistant, Mr. Cambier, as well as upon animals. It was likewise shown that the proteid, which was extracted from the bark by cold water, is coagulated by heat, and that its toxic properties thereby become completely destroyed. This served to explain why in some of the preliminary experiments for the isolation of the poisonous principle a considerable amount of an extract of the bark, prepared by the aid of heat, could be taken by us without any ill-effects. The isolation of the poisonous proteid seemed furthermore of particular interest, inasmuch as it was believed to be the first, and is as yet, apparently, the only recorded observation of the occurrence of such a body in a bark, the other allied substances, such as ricin, abrin, and croton, being found in the seeds of the respective plants.

With the advance in knowledge of the proteid substances it seemed to me desirable to again take up this investigation, and at the same time to examine more closely the other constituents of the bark.

The present publication, fortunately, also affords an opportunity of considering some statements in recent German literature pertaining to this subject, from which it would appear that the results of my previous investigation have been to some extent overlooked, and that incorrect deductions have thereby been formed. My attention was first directed to this by a short communication from Professor Kobert to the *Apotheker Zeitung* of May 25, 1901, p. 365, with reference to a note published a short time previously by Professor E. Schmidt (*loc. cit.*, 1901, p. 357) on "Robinin"—a name given in 1861 by Zwenger and Dronke to a colouring matter obtained by them from the flowers of *Robinia Pseud-acacia*. An abstract of Professor Kobert's observations may be given in the following translation:—"It appears to me in the interest of your readers to point out that in medical literature the word *Robinin* has hitherto been used in another sense. In a few days there will appear in Rostock a dissertation of my pupil, Dr. Carl Lau, 'Ueber vegetabilische Blutagglutinine,' in which there will be a report on the Robinin of medical authors and a proposal to change the name of this to *Robin*, since it possesses an action very similar to abrin, ricin, and croton, but weaker

Robin was first described under the name of *Robinin* by Power and Cambier (*Pharm. Journ.*, 1890, p. 711). They found this substance, designated by them as a *phyt-albumose*, to the extent of 1·6 per cent. in the bark of *Robinia Pseud-acacia*."

Such a confusion of names would naturally be unfortunate, and I was not aware that it existed, since in my original paper and in the abstract cited no mention whatever was made of the name "*Robinin*" in connection with the toxic proteid. In a private communication from Professor Kobert he has kindly informed me that the use of the word *Robinin*, which was incorrectly attributed to me, has occurred through inaccurate abstracts in medical literature.

In the above-mentioned dissertation of Dr. Carl Lau (Rostock, 1901), for a copy of which I am indebted to Professor Kobert, there are some passages relating to the *Robinia* proteid which it would seem desirable to note in this connection in order that some comment may be appended to them. Thus on pages 16-17 it is recorded:—"Als viertes pflanzliches Blutagglutinin ist das *Robin* (früher *Robinin*) zu nennen, welches vor Jahren von Professor Kobert als solches erkannt, bis jetzt weder vom Entdecker noch von jemand anderem genauer als solches beschrieben worden ist. Power und Cambier (*Pharm. Journ.*, 1890, p. 711) fanden einen als *Robinin* bezeichneten Stoff zu 1·6 per cent. in Form einer *Phytalbumose* in der Rinde der *Robinia pseudacacia* neben einem Alkaloid, welches Aehnlichkeit mit *Cholin* und *Neurin* hat. Ich möchte in ersterer Substanz unser *Robin* erblicken *Robinia pseudacacia* ist wissenschaftlich recht wenig untersucht worden."

In consideration of the above statements it will be sufficient to refer those interested in the subject to my original publication (*loc. cit.*).

On page 28 the author appends the following note:—"Ich habe in diesem Falle nicht, wie bei *Abrin*, *Ricin* und *Crocin* eine 1 % ige, sondern eine 5 % ige *Robinlösung* verwandt weil ein grosser Theil des *Merck'schen Präparats* wegen seines Alters—es stand schon 12 Jahre bei Prof. Kobert—unlöslich geworden war."

There is apparently a slight error in this statement, according to which it would appear that *Robin* had been prepared by Merck at least twelve years ago, whereas there is no evidence that the substance in question was known prior to the publication of my paper in 1890.

On page 40, in connection with physiological experiments for determining the toxic action of *Robin*, Dr. Lau remarks as follows:—"Ich würde sehr gern noch eingehendere Versuche darüber

angestellt haben, ob die giftige Eiweisssubstanz der Robinienrinde ein Albumin, eine Albumose, oder ein Globulin, oder ein Gemisch zweier Substanzen ist. Zu derartigen Versuchen hätte ich jedoch viel grössere Mengen von Material gebraucht als sie mir zur Verfügung standen. Ich musste mich daher damit begnügen festgestellt zu haben dass es sich thatsächlich um eine giftige Eiweisssubstanz handelt."

From the latter statement one would naturally be led to infer that the toxic action of the Robinia proteid had only become established as an actual fact by these recent experiments, whereas this would seem to have been sufficiently demonstrated more than eleven years ago. It appears the more remarkable that this should have been overlooked in view of the fact that in acknowledgment of a specimen of the proteid sent to the late Professor Flückiger, of Strassburg, he wrote me under date of February 4, 1892, as follows:—"I have to thank you for the poison of Robinia, which I sent finally to Prof. Kobert, Dorpat (Russia). He has also prepared the poison, and states now that it nearly agrees with your preparation."

At the close of his dissertation, p. 63, Dr. Lau likewise refers to the previously mentioned crystalline colouring matter obtained from Robinia flowers, termed *Robinin*, which has been under investigation by Professor A. G. Perkin (*Proc. Chem. Soc.*, 1900, No. 219, p. 45; 1901, No. 236, p. 87), and is at present also being examined by Professor E. Schmidt (*Apotheker Zeitung*, 1901, p. 357), and remarks as follows:—"Man wird daraus ersehen, wie zeitgemäss es war, unsern Giftstoff aus der Robinie in Robin umzubenennen."

It is evident that the name *Robin*, as applied to the toxic proteid of Robinia bark, is a very appropriate one, and it need finally only be observed that this designation has, in fact, been employed by Ehrlich in his physiological investigations, to which I shall subsequently refer, as also in pharmaceutical literature by E. Schmidt ('Pharm. Chemie,' third edit., 1896, Bd. ii., p. 1,647), who refers to it as follows:—"Als Robin wird das in der Rinde von Robinia Pseud-acacia enthaltene, stark giftige Toxalbumin bezeichnet."

Chemical Examination of Robinia Bark.

(1) THE TOXIC PROTEID, OR ROBIN.

In the first chemical examination of this bark the material was collected by myself in the neighbourhood of Madison, Wisconsin, where the tree is largely cultivated, and only the light-coloured inner bark was employed. The material for the present

investigation, which was quite the same in its character, was obtained from France, and had been freshly collected.

Preparation of the Proteid.—The toxic proteid was first obtained by adding to a cold, concentrated, aqueous infusion of the bark a considerable quantity of strong alcohol, and actively shaking, when a voluminous flocculent precipitate is produced. After standing for several hours, the precipitate was collected on a filter, and washed with a little alcohol. It then forms a nearly white, slimy mass, which, when spread on plates of glass and dried in a vacuum desiccator over sulphuric acid, is obtained in the form of yellowish-brown scales. The yield of dry substance was 1.66 per cent. of the weight of the bark. In the present investigation the yield of proteid, prepared in practically the same manner, was 1.14 per cent. In both cases the air-dry, coarsely-ground bark was used, but the yield of proteid will naturally depend upon the completeness of its precipitation, and probably also upon the season at which the bark is collected, the late spring or early summer being doubtless the most favourable period.

The proteid may also be prepared by extracting the ground bark with a 10 per cent. solution of sodium chloride, and subsequently saturating the filtered liquid with ammonium sulphate. The precipitate is then spread on plates of glass to dry. By this method it is associated with a considerable amount of ammonium sulphate, which may be removed by dialysis, and the filtered dialysed liquid may then be precipitated by alcohol; but during the operation a considerable portion of the proteid is rendered insoluble. For the experiments here described the proteid was prepared by simply allowing the ground bark to stand for about twenty-four hours in contact with sufficient cold water to cover it, expressing, filtering, and adding to the filtrate a large volume of strong alcohol.

Characters of the Proteid.—The precipitated proteid, either while still moist or when dried, does not again dissolve completely in water, but is rendered soluble by the addition of a little alkali. A specimen dried over sulphuric acid afforded (i.) 4.12, (ii.) 3.74 per cent. of ash; it would be difficult to determine to what extent this represents an actual impurity, inasmuch as certain inorganic elements appear to form an integral part of the complex molecule.

The solution of the proteid has an acid reaction. It is precipitated by the mineral acids, and, especially when acidulated, by all the usual alkaloidal reagents, such as potassium-mercuric iodide, potassium-bismuth iodide, iodine solution, phosphomolybdic acid, tannic acid, picric acid, mercuric chloride, and

by copper acetate and potassium ferrocyanide, as also by ferric chloride and the neutral and basic acetates of lead.

The proteid affords the various colour reactions characteristic of this class of substances, such as the following:—(1) The so-called biuret reaction, by adding to the aqueous solution of the proteid a solution of caustic alkali and a few drops of a dilute solution of copper sulphate, when a handsome bluish-violet colour is produced. (2) The xanthoprotein reaction, or deep yellow colour produced in the solution by strong nitric acid. (3) Millon's reaction, with a solution of mercurous nitrate, which produces a rose-red precipitate. Although this reaction is obtained with all the natural albumens, it is stated to be afforded only by such of the albumoses as belong to the *hemi* group. (4) Molisch's reaction, with *α*-naphthol and sulphuric acid, which produces a violet colour. (5) Liebermann's reaction, by heating a little of the dry proteid with concentrated hydrochloric acid, when a bluish-violet colour is produced. As both this action and the preceding one are recognised as furfural reactions, they are supposed to indicate the presence of a carbohydrate group in the proteid complex.

The reaction of Adamkiewicz, produced by the action of concentrated sulphuric acid upon a solution of the proteid in glacial acetic acid, could not be obtained in the present instance, but this may be easily explained by the recent observations of Hopkins and Cole (*Chemical News*, February, 1901, pp. 73, 85), who have shown that the production of the particular colour is dependent upon the presence in the acetic acid of a small amount of an impurity, which they found to be glyoxylic acid, $\text{CH}(\text{OH})_2$.

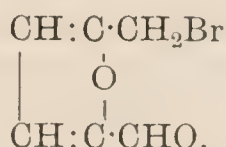


Such specimens of acetic acid as do not contain this impurity, or at least not in sufficient amount, will therefore give a negative result.

In order to ascertain whether a sugar could be obtained from the proteid by hydrolysis, one gramme of the latter was heated in a sealed tube with 25 C.c. of 5 per cent. sulphuric acid for eight hours at 100° C. The liquid was then filtered, digested with barium carbonate for the removal of the free acid, and decolorised with animal charcoal. It was found to be slightly dextro-rotatory, and reduced Fehling's solution, but gave only a small amount of a rather unsatisfactory osazone, melting at about 146°-147° C.

With consideration of the interesting investigations of Fenton and Gostling on "the action of hydrogen bromide on carbohydrates" (*Journ. Chem. Soc.*, 1898, 73, p. 554, and 1901, 79.

p. 361), in which it has been shown that certain classes of carbohydrates when acted upon at the ordinary temperature with dry hydrogen bromide in ethereal solution give an intense purple colour, and suggested that the rapid production of this colour was indicative of *keto*hexoses, or of substances which produce them by hydrolysis, it was thought of interest to try the action of this reagent upon the Robinia proteid. It has been noted, however, by Fenton in his most recent communication that the production only of a purple colour is not sufficiently conclusive, and that the observation should be confirmed by the actual isolation and identification of crystals of the respective compound ω -bromo-methylfurfural, $C_6H_5O_2Br$, or



The method he has suggested as most suitable for quantitative purposes was therefore adopted in the following experiment. One gramme of the proteid and 12.5 C.c. of chloroform, which had been saturated at 0° C. with dry hydrogen bromide, were heated together in a sealed tube at the temperature of the water-bath for two hours. An insignificant purplish colour was produced on the surface of the tube around the substance, but even after standing for several hours, this colour was not imparted to the liquid, whilst the substance itself had become quite black. The chloroform solution was then treated successively with anhydrous sodium carbonate and a few drops of a strong solution of this salt, dried over calcium chloride, and finally allowed to evaporate in a weighed glass dish. A very slight residue was obtained, which contained no crystals, from which it may be inferred that a ketohexose grouping of the carbohydrate complex does not exist in the proteid molecule.

In order to ascertain whether the Robinia proteid, or any substance associated with it, is capable of acting as an enzyme or ferment the following experiments were made, which have been attended with interesting results.

To an aqueous solution of amygdalin, contained in a test tube, a small amount of the dry, precipitated proteid was added, the mixture well shaken, and the tube corked and set aside. After standing for a short time at the ordinary summer temperature a strong odour of bitter almond oil was developed, and the filtered liquid gave an abundant reaction for hydrocyanic acid. The amygdalin had thus been split up in the same manner as by emulsin, and it may be noted that this decomposition had previously been observed to be effected by the ferment (myrosin)

contained in a cold infusion of white or black mustard seed (Flückiger, *Pharm. Chemie*, 2nd edit., pt. ii., p. 10), as also by diastase (Robiquet) and beer yeast (Ronke). (Compare Husemann, *Die Pflanzenstoffe*, 2nd edit., p. 1020).

A little of the proteid was then added to an aqueous solution of pure potassium myronate, when, after standing for a short time under the same conditions, a strong odour of mustard oil was developed. This result appears to be of particular interest, inasmuch as emulsin and beer yeast are stated to be incapable of effecting the decomposition of potassium myronate, and the Robinia ferment would therefore more closely resemble myrosin than emulsin. Whether this fermentative action represents a function of the toxic proteid, or whether it is due to a substance associated with it, is a question that must at present remain undecided. The occurrence of such a ferment indicates, moreover, that it stands in some genetic connection with a glucoside existing in the bark, which will be described later on.

In his recently published dissertation (*loc. cit.*) Dr. Carl Lau has recorded some experiments with the toxic proteids, ricin, abrin, and crotin, which were undertaken in order to ascertain whether these bodies possess the property of agglutinating other cellular elements besides the red corpuscles of the blood. It was thus found, for example, that when allowed to act upon either boiled or unboiled milk, under definite conditions, they are capable of coagulating the casein in a manner similar to that of the rennet enzyme. As the Robinia proteid was apparently not included in these experiments, it was thought desirable to subject it to corresponding tests, which were conducted as follows:—Into four test tubes, marked respectively *A*, *B*, *C*, and *D*, were brought the following liquids:—*A* contained 10 C.c. of fresh milk. *B* contained 10 C.c. of milk with 1 C.c. of a 5 per cent. solution of Robin in water. *C* contained 10 C.c. of milk with 1 C.c. of a 5 per cent. solution of Robin in 10 per cent. solution of NH_4Cl . *D* contained 10 C.c. of milk with 5 C.c. of a concentrated infusion of Robinia bark. A similar series of tubes was prepared with the use of freshly boiled milk. All of the tubes were then placed in water kept at a temperature of $35^\circ\text{--}40^\circ\text{C}$. In about fifteen minutes the unboiled milk in tubes *B*, *C*, and *D* began to curdle, and after one hour the corresponding tubes containing boiled milk began to change. After standing for six hours the same tubes of both boiled and unboiled milk were considerably curdled, the change being most marked in the latter. The tubes *A*, containing both boiled and unboiled milk, which served for the purpose of control, remained quite unchanged. From these results it is evident that the Robinia proteid is like-

wise capable of effecting changes similar to those produced by the rennet enzyme.

The exact position which should be assigned to the so-called *Robinia proteid* or *Robin* among the various groups of related substances is somewhat difficult to determine, partly on account of the different methods of classification or differences of nomenclature adopted by various investigators. With consideration of the deportment of these bodies, as described in the recent work of Cohnheim, entitled *Chemie der Eiweisskörper*, Braunschweig, 1900, it would appear to belong to the group which has received the distinctive designation of *Proteids*, and possibly to the division of so-called *nucleo-proteids*. It may be noted, however, that the term "proteid" is employed by German chemists in a much more restricted sense than in this country or in France, where it is commonly understood to include all albuminous substances. Although Robin has some of the properties both of an albumose and of a globulin, it differs, on the other hand, from these in being soluble in water, in being completely coagulated by heat, and in some of the reactions which are accepted as characteristic of them. It appears, moreover, quite probable that the Robinia proteid consists of more than one albuminous body, for it has been observed that a saline solution extracts a larger amount of proteid from the bark than water alone, and, in addition to its previously described characters, including those of an enzyme, by carefully heating its solution it may be resolved into fractions having different coagulating points.

Physiological Action of the Proteid.—The most recent investigation of the physiological action of Robin is that included in the dissertation of Dr. Carl Lau (*loc. cit.*), who has shown that it has the property of agglutinating the red corpuscles of the blood of various animals, but that it has not this effect upon human blood or upon that of the dog and cat. Its action in this respect is also less intense than that of ricin, abrin, and crotin. Experiments on animals, chiefly rabbits, by hypodermic injections of a solution of Robin, have shown that its toxic action is attended by an alteration of the kidneys, often producing a nephritis, or at least manifested by the presence of more or less albumen, hæmoglobin, and cylindrical casts in the urine.

In this connection it would seem desirable to refer very briefly to some observations of Ehrlich, which relate to the immunising property of the Robinia proteid. In his paper entitled "Die Wertbemessung des Diphtherieheilserums und deren theoretische Grundlagen" (*Klinisches Jahrbuch*, Bd. vi., 1898, p. 315), in which he considers the genesis of the toxoids which are found in relatively large quanti-

ties in long-kept diphtheria poisons treated with preserving media, Ehrlich remarks as follows:—"In the case of the vegetable toxalbumins (ricin, abrin, *robin*, crotin) there are also positive indications of the presence of toxoids. The fact appears to me to be specially worthy of notice that the anti-toxin produced by robin—the toxalbumin of *Robinia pseud-acacia*—corresponds almost entirely in its properties to anti-ricin, notwithstanding that the two initial bodies—robin and ricin—are certainly different. *These conditions indicate that the much less poisonous robin represents a naturally occurring toxoid of ricin.*" It may be explained that Ehrlich employs the term "toxoid" to designate a modified toxin, but he refrains from any hypothesis regarding their formation.

In a paper by George Ogilvie, B.Sc., M.R.C.P., entitled, "Some Remarks on the Inheritance of Acquired Immunity" (*British Medical Journal*, May, 1901, p. 1,070), the following further observation is recorded:—"Ehrlich's ingenious experiments—made on mice with the vegetable toxins of ricin, abrin, and *robin*—have led him to the conclusion that no trace of immunity is ever conferred by parent upon offspring through the germinal cells, either by the sperm or by the ovum."

(2) OTHER CONSTITUENTS OF THE BARK.

As a preliminary test for confirming the presence of organic bases, which a previous investigation had indicated to be present, 20 grammes of the ground bark were digested for several days with 100 C.c. of Prollius' fluid. The filtered liquid left on evaporation a slight amorphous residue, which, when taken up with acidulated water, afforded abundant reactions with all the usual alkaloidal reagents.

100 grammes of the ground bark were then extracted successively by various solvents, in the following order:—(1) *Light petroleum* (b.p. 40-60° C.).—This afforded 1.15 per cent. of fatty matter, which, when treated with acidulated water, gave no alkaloidal reaction. (2) *Ether*.—This afforded 0.4 per cent. of a brownish resin, which gave no alkaloidal reaction. (3) *Chloroform*.—This afforded 0.2 per cent. of a brownish resin, which, when extracted with acidulated water, gave a slight alkaloidal reaction. (4) *Alcohol*.—The concentrated, very dark coloured alcoholic liquid was poured into water, in order to precipitate resinous matter. The aqueous filtrate was abundantly precipitated by alkaloidal reagents. It slightly reduced Fehling's solution, but to a much greater extent after heating with a little hydrochloric acid, when a peculiar, somewhat

aromatic odour was developed, thus indicating the presence of a glucoside.

A larger quantity of the bark was now operated upon as follows:—Two kilos of the ground bark were extracted first with cold, and then with hot alcohol. After distilling off the alcohol the liquid was poured into water, and the aqueous liquid filtered from the precipitated fatty matter and resin. The character of the latter bodies will be considered later.

The aqueous liquid was treated with lead acetate for the removal of colouring matter, filtered, and the excess of lead removed by hydrogen sulphide. It was now found to be precipitated by all the usual alkaloidal reagents, and was subsequently evaporated to a thick syrup, and extracted in a Soxhlet with strong alcohol. To the dark coloured alcoholic solution a saturated alcoholic solution of mercuric chloride was added, which produced a whitish precipitate, soon changing, however, to a very dark, resin-like mass. After standing for a time, the clear liquid was decanted, and to the latter another portion of mercuric chloride was added, which produced a similar precipitate. To the liquid decanted from the second precipitate a third portion of mercuric chloride was added, with the same result as before. The three precipitates (*a*), (*b*), and (*c*), after being washed with a little alcohol, were extracted with warm water, when in each case a considerable amount of a dark-coloured amorphous substance remained undissolved. The filtered liquids were all separately treated with hydrogen sulphide to remove the mercury and again filtered.

(*a*) This was a dark-coloured liquid, which was treated with animal charcoal, then made alkaline with sodium hydrate, and shaken out five times successively with hot amyl alcohol. The latter was then shaken with water, acidulated with hydrochloric acid, when dense white fumes were produced, probably due to ammonia. The acid liquid gave all the characteristic alkaloidal reactions, and, after treatment with animal charcoal, was obtained nearly colourless. It was allowed to evaporate in a vacuum desiccator over potash and sulphuric acid, but, as it became concentrated, it acquired an almost black colour, showing that decomposition had ensued. It was, however, taken up with a little absolute alcohol, treated with animal charcoal, and the solution precipitated by platinic chloride. 0.0360 gramme of this salt gave on ignition 0.0160 gramme of platinum or 44.4 per cent. Pt. The precipitate, therefore, consisted simply of ammonium-platinic chloride, which requires 43.9 per cent. Pt.

The liquid, after extraction with the hot amyl alcohol, when

acidulated, still afforded a strong alkaloidal reaction, but it was evident that during contact with the alkali the basic substance was being continually decomposed with the evolution of ammonia.

(b) This liquid was treated in the same manner as described under (a), and, although originally nearly colourless, when allowed to evaporate in a vacuum desiccator it afforded an almost black residue. The latter, when treated with absolute alcohol, left a crystalline residue of ammonium chloride, and from the alcoholic solution a small amount of a platinum salt was obtained, which, on analysis, also gave figures agreeing with those required for ammonium chloride. 0.0405 gramme of the salt gave on ignition 0.0180 gramme of platinum, or 44.4 per cent. Pt.

(c) This liquid, which seemed to be the purest of the three fractions, was treated in the same manner as the two preceding ones. The residue left on evaporation contained some crystals of ammonium chloride, and the platinum salt obtained from the alcoholic solution was analysed with the following result: 0.056 gramme of the salt gave on ignition 0.021 gramme of platinum or 37.5 per cent. Pt. It is possible, therefore, that this fraction contained one of the methylamines, or a mixture of these bases. The amorphous brown residues which remained on treating the alcoholic mercuric chloride precipitates with water were suspended in water and decomposed by hydrogen sulphide. The filtrate was very dark in colour, gave precipitates with alkaloidal reagents, and also developed ammonia when heated with a caustic alkali. On evaporation it left simply a dark amorphous residue.

The alcoholic liquid remaining after precipitation with alcoholic mercuric chloride was treated with hydrogen sulphide to remove the excess of mercury, and filtered. After distilling off the alcohol, a little water was added, and the liquid further evaporated, when a peculiar disagreeable odour was developed, and a considerable amount of a black resin separated. After purifying the very dark-coloured liquid by means of lead subacetate it still afforded a strong alkaloidal reaction. It was finally made strongly alkaline with potassium hydrate, distilled, and the distillate collected in water acidulated with hydrochloric acid. On evaporating the acid distillate to dryness, and treating the residual salt with absolute alcohol, a considerable amount of ammonium chloride was left undissolved. The alcoholic solution, after evaporating and again taking up the residue with alcohol, afforded a small amount of a platinum salt which was analysed with the following result: 0.0772 gramme of the salt

gave on ignition 0.03 gramme of platinum, or 38.86 per cent. Pt. The platinumchloride of dimethylamine contains 38.98 per cent. of platinum, but it is possible that the salt examined consisted of a mixture of amines. The results obtained, however, clearly demonstrate that the organic bases contained in the original liquid easily become decomposed by the method employed for their isolation, and especially by the action of caustic alkalies, with the evolution of ammonia, and apparently one or more amines.

A similar result was obtained by extracting a kilo of the ground bark with acidulated water, and subsequently making the liquid strongly alkaline with potassium hydrate and distilling. The distillate had a strongly ammoniacal, but also distinctive odour, and, when neutralised with hydrochloric acid, afforded on evaporation about 9 grammes of a salt consisting chiefly of ammonium chloride. On treating this with alcohol, and fractionally precipitating with platinic chloride, salts were obtained which gave respectively 38.88 per cent., 40.32 per cent., and 40.48 per cent. of platinum.

Two other portions of bark of 1 kilo each were extracted, one with cold water, and the other with acidulated water, then mixed with milk of lime and separately distilled. From each portion about 13 grammes of dry salt were obtained, consisting almost entirely of ammonium chloride. The extremely small amount that was finally soluble in absolute alcohol afforded platinum salts containing respectively 42.10 per cent., and 42.46 per cent. of platinum, which indicated that these were also mixtures.

The large amount of ammonia produced in the last-mentioned experiments is naturally due to the direct decomposition of the soluble proteid by the caustic alkali.

For the further examination of the constituents of the bark, with the hope of determining not only the character of the substance affording the alkaloidal reactions, but also the nature of the glucosidal body whose existence had been indicated, a larger amount of material was operated upon in the following manner.

Seven kilos of the ground bark were extracted with hot alcohol in a continuous extraction apparatus, and the alcohol for the most part distilled off. The dark-coloured liquid thus obtained was mixed with water to separate fatty and resinous matter, and the liquid filtered. The filtered liquid was then treated with basic lead acetate, which produced a dense precipitate, consisting chiefly of tannic and colouring matter, but apparently nothing of further interest. The filtrate from the lead precipitate was treated with hydrogen sulphide, and again filtered, when it had a bright yellow colour. It was now concentrated under

diminished pressure, in order to avoid any darkening in colour, until it acquired an almost syrupy consistence. On further evaporation it formed a thick syrup, but without a distinctly sweet taste. A small portion which had been allowed to stand for a considerable length of time deposited a few small, needle-shaped crystals, which could not be separated from the syrupy liquid, but which will be referred to later. The liquid shows the following behaviour toward reagents. It is precipitated by all the usual alkaloidal reagents, and more abundantly when acidulated with hydrochloric acid. When heated with an alkali hydrate it evolves ammonia, at the same time developing a peculiar odour, reminding somewhat of methylamine. It slightly reduces Fehling's solution, but much more strongly after heating with hydrochloric acid. The original solution was dextro-rotatory, and afforded an osazone melting at 197° C.

The entire liquid was now divided into two equal parts, which may be designated as *A* and *B*.

(A) To this portion of the liquid an aqueous solution of mercuric chloride was added. A light-coloured precipitate was thus formed, which continued to redissolve until a considerable excess of the reagent was added. The precipitate was finally collected on a filter, washed with a little water, in which it was somewhat soluble, then suspended in water and decomposed by hydrogen sulphide. The filtrate from this decomposed precipitate was of a deep red colour, and was allowed to evaporate in a vacuum desiccator, when a very dark-coloured residue was obtained, which was taken up with a little absolute alcohol. This alcoholic solution afforded with platinic chloride only a small amount of a resin-like precipitate, which was not suitable for further examination.

The filtrate from the original mercuric chloride precipitate was then treated with hydrogen sulphide, for the removal of the excess of mercury, and filtered. To the strongly acid liquid, which still gave the alkaloidal reactions as strongly as before, an additional 10 C.c. of hydrochloric acid were added, and it was then boiled in a flask provided with a reflux condenser for about three hours. The liquid, when cold, was filtered from a considerable amount of black resinous substance, and distilled. The distillate, which had a peculiar aromatic odour, was shaken out with ether, the latter separated and allowed to evaporate, when a small amount of an oily residue was obtained, having an aromatic, vanilla-like, but at the same time somewhat empyreumatic odour. It gave no reaction with ferric chloride. The strongly acid liquid remaining in the flask was again filtered, and shaken several times successively with ether. The ethereal liquids were

of a reddish colour, and when evaporated left a dark-red oily residue. On treating this with water a small amount of white needle-shaped crystals were separated. These were collected on a filter, and after washing with a little chloroform and ether, in which they were not very freely soluble, they were obtained quite white. On subsequently shaking the acid liquid with several successive portions of chloroform, more of the red amorphous substance was obtained, from which, by means of water, a small additional amount of nearly colourless crystals was obtained.

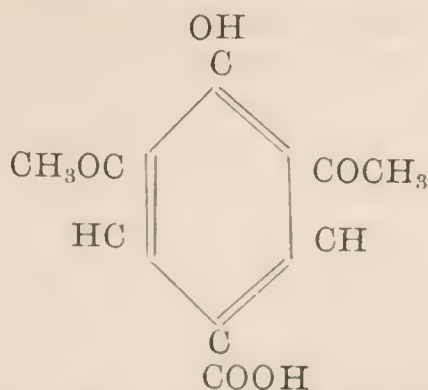
The purified crystals, extracted by means of ether, formed white, silky needles, and contained no nitrogen. They melted sharply at 198° - 199° C. (corr.). The crystals have an acid reaction to test paper, and when brought into a little solution of caustic potassa or soda they dissolve with a rose-red colour, which soon fades. Nitric acid produces a deep red colour, soon changing to yellow, and ferric chloride produces a slight brown coloration.

When dried in a water-oven at 100° C. the crystals lost nothing in weight, and were then analysed, with the following result:—0.0422 gramme of substance gave 0.0836 gramme CO_2 and 0.0194 gramme H_2O .

Calculated for $\text{C}_9\text{H}_{10}\text{O}_5$.	Found.
C = 54.5 per cent.	54.0 per cent.
H = 5.1 per cent.	5.1 per cent.

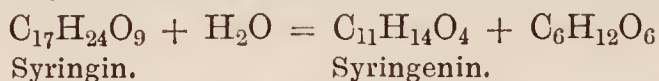
During the combustion the substance was observed to gradually sublime, forming glistening, silky crystals on the cooler part of the tube.

Although the amount of pure substance was only sufficient for one analysis, the figures obtained are observed to agree remarkably well for a body of the composition $\text{C}_9\text{H}_{10}\text{O}_5$, which is that of *syringic acid*, or the 3.5 — dimethyl ether of gallic acid.

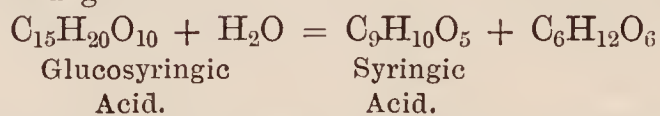


There are, moreover, some other facts which serve to establish the identity of this substance as syringic acid. In the first place,

its melting point is given in Beilstein's 'Handbuch der organischen Chemie,' Bd. ii., p. 1,921, as 202° C., which, being probably uncorrected, would agree very well with that observed. In the second place, it was obtained from a substance, evidently a product of hydrolysis, forming an amorphous, rose-red mass, which would therefore correspond in its properties to *syringenin*, a body which, together with glucose, is formed from the glucoside *syringin* by heating with dilute mineral acids, thus:—



It is known that syringin, when oxidised, is converted into *gluco-syringic* acid, and that the latter by hydrolysis forms syringic acid and glucose.



This would easily explain in the present instance the occurrence of the small amount of syringic acid which was found associated with the syringenin in the products of hydrolysis. It naturally also indicates the occurrence in Robinia bark of the glucoside *syringin*, which was first found in the bark of *Syringa vulgaris*, and subsequently in the bark of *Ligustrum vulgare* (compare van Rijn, 'Die Glykoside,' p. 356). It is probable that the few crystals which were observed to have been formed in a small portion of the original liquid on standing for some time, as previously noted, consisted of this initial substance, although they could not easily be isolated from the thick syrup. The fact that the crystals of syringic acid did not pre-exist in the original liquid, but that they were a product of hydrolysis, was also quite satisfactorily proved by extracting a portion of the bark with hot water, purifying it from colouring matter by means of lead subacetate, and shaking out with ether, when no crystalline substance could be obtained. It was also confirmed by the examination of another product, which will be described further on.

The liquid from which the syringic acid had been obtained had a strongly reducing action upon Fehling's solution, and was dextro-rotatory. From a small portion of it an osazone was prepared which melted at 205-206° C. The melting point of *d*-glucosazone is 202° C., and the hydrolysed liquid therefore contained a considerable amount of *d*-glucose.

It was noted in connection with the *Robinia* proteid, in the first part of this paper, that either this body or a substance associated with it has the properties of an enzyme, which is in accordance with the view that these principles accompany glucosides in plants. It was furthermore noted that this enzyme resembles myrosin, the ferment contained in mustard seed,

in its properties, inasmuch as, like the latter, it not only acts upon potassium myronate with the formation of mustard oil, but also develops hydrocyanic acid and benzaldehyde from amygdalin. It therefore appears to be an interesting fact that sinapin, which occurs ready formed in black mustard seed, and which is likewise formed from the sinalbin contained in white mustard seed through the action of the ferment myrosin, is converted by the action of alkalis into choline and sinapinic acid, and that the latter body is assumed to stand in direct relation to syringenin, syringenin being regarded as the alcohol of sinapinic acid (compare van Rijn, *loc. cit.*, p. 108). This naturally suggests the possibility of some intimate organic connection between the enzyme and the glucoside of Robinia bark.

As the hydrolysed liquid, which had been extracted by ether, still afforded a strong reaction with alkaloidal reagents, and as it had previously been found that the body producing these reactions could not be extracted by immiscible solvents, either from acid or alkaline solutions, the entire remaining liquid was precipitated by potassium-bismuth iodide. This afforded a considerable amount of a flocculent, brick-red precipitate, which was collected on a filter, washed with a little acidulated water, then suspended in water, and decomposed by hydrogen sulphide. The filtrate, after treatment with a little animal charcoal, was shaken with freshly precipitated silver hydroxide for the removal of the hydriodic acid, and was then rapidly filtered, as it was observed that even by contact with the silver hydroxide the organic base was gradually becoming decomposed with the evolution of ammonia or an amine, for dense white fumes were produced in contact with the vapour of hydrochloric acid. A few drops of hydrochloric acid were then added to precipitate the small amount of dissolved silver hydroxide and convert the base into an hydrochloride, again filtered, and the slightly coloured liquid allowed to evaporate in a vacuum desiccator over caustic potash and sulphuric acid. As there was no indication of a crystalline salt being formed, the concentrated solution was at once precipitated by gold chloride. The precipitated aurichloride, which was amorphous and of a light yellow colour, was quickly transferred to a filter, washed with a little water, and dried. An attempt to crystallise it from alcohol was unsuccessful. It melted somewhat indefinitely at about 117° C., and was analysed with the following result:—(1) 0.1030 gramme of the salt gave 0.0392 gramme of gold, or 38.06 per cent. Au. (2) 0.1982 gramme of the salt gave 0.0754 gramme of gold, or 38.04 per cent. Au.

A more complete analysis of the salt was considered unnecessary, inasmuch as it had been prepared from an indefinite substance, or possibly mixture of bases, which could not be obtained in a crystalline form. The substance in question, however, was evidently not choline, which the results of a previous investigation had led me to believe might be present in the bark, since choline aurichloride would require 44.4 per cent. of gold. As in the former instance a platinum salt was employed for analysis, an error had probably been introduced through the hitherto unobserved formation of ammonia by the action of the silver hydroxide. The observed alkaloidal reactions cannot be due to such bodies as the amino-acids—glycocoll, leucine, and asparagine, or to tyrosine, since they are not precipitated in acid solution by alkaloidal reagents, nor do they evolve ammonia in contact with an aqueous alkali. On the other hand, it is quite probable that the reactions must be attributed to some basic degradation products of the proteid, which cannot as yet be more exactly defined, and which themselves readily undergo further decomposition into simpler substances.

(B) This second portion of the original liquid, freed from colouring matter by means of lead subacetate, was precipitated by a concentrated solution of tannic acid. On the first addition of the reagent a whitish precipitate was formed, which soon changed to a resinous, sticky mass, and became redissolved to a considerable extent until a large excess of tannin had been added. On account of its solubility in the tannin solution a complete separation of the substance thus precipitated could not be effected. The liquid, however, was decanted, the precipitate washed with a little water, and subsequently treated with hot water, in which it was soluble. This hot solution was mixed with lead carbonate, and the mixture repeatedly evaporated on a water-bath until the liquid was free from tannin. The dry powder was then brought into a Soxhlet and extracted with strong alcohol. A dark-coloured liquid was thus obtained, which was allowed to evaporate spontaneously, when a thick, uncrystallisable syrup remained. Its behaviour towards alkaloidal reagents and caustic alkalies was the same as has been previously described, and it was not considered of further interest.

The filtrate from the above-mentioned tannin precipitate was treated with lead subacetate and afterward with lead carbonate for the removal of the tannin, and the lead then removed by hydrogen sulphide and filtered. This filtrate, which had a pale yellow colour, was concentrated under diminished pressure,

and placed in a vacuum desiccator. About 75 grammes of a very thick, pale yellow syrup were finally obtained, from a portion of which, after standing for a considerable time, a few colourless crystals were observed to separate, which possibly consisted of the glucoside syringin, but they could not be separated. The syrup, when diluted with a little water, was found to be strongly dextro-rotatory, and was not precipitated by potassium-mercuric iodide, but by some of the other alkaloidal reagents. It was shaken out with several portions of chloroform, but the latter liquids on evaporation left simply a very small amount of an oily residue, from which water extracted no crystalline substance.

The syrupy liquid was then oxidised at the ordinary temperature with a cold saturated solution of potassium permanganate with the hope of obtaining the so-called *gluco-syringic acid*, $C_{15}H_{20}O_{10}$, since this substance, as previously observed, is obtained by the oxidation of syringin, and by hydrolysis is converted into syringic acid and glucose. Among the products of oxidation, however, there was found only *oxalic acid*, and, by extracting the acidulated liquid with ether, a crystalline substance which, after re-crystallisation from glacial acetic acid, melted at $185^{\circ} C.$, and had all the properties of *succinic acid*.

With the same purpose in view of obtaining, if possible, the above-mentioned glucoside acid, an extract was prepared from about 250 grammes of Robinia bark by boiling it with water, and purifying the liquid by means of basic lead acetate. When concentrated under diminished pressure it formed a thick syrup, which did not reduce Fehling's solution until after boiling with hydrochloric acid. The syrupy liquid was oxidised with cold potassium permanganate, as above described, and the acidulated liquid extracted several times with ether. The ethereal solution, which had a strong smell of acetic and the higher fatty acids, likewise afforded chiefly *oxalic and succinic acids*. The succinic acid, after being recrystallised from glacial acetic acid, was readily identified, not only by its qualitative reactions, but by its melting point of $184^{\circ} C.$, and by the analysis of its silver salt, which gave the following figures:—0.0743 gramme of the salt gave, on ignition, 0.0481 gramme of silver, or 64.7 per cent. Ag. $Ag_2C_4H_4O_4$ requires 65.1 per cent. Ag.

THE RESINS.

In the first part of this paper it was noted that the original alcoholic extract of the Robinia bark contains a considerable amount of resin and fatty matter, which was separated by the

addition of water. This crude product was treated first with petroleum spirit, which extracted a quantity of very dark coloured, soft resin and fatty matter. The portion undissolved by the petroleum spirit was dissolved in warm alcohol, and the concentrated solution poured into a large volume of water acidulated with sulphuric acid, when the resin separated in a flocculent form, and, after being washed and dried, could be reduced to a fine, brown powder. The yield of soft resin was 86 grammes, or 1.23 per cent. of the weight of the bark; the yield of dry, powdered resin was 37 grammes, or 0.53 per cent. of the weight of the bark.

After distilling off the alcohol from that portion of the above crude resin that had been dissolved in it, it was observed that the liquid in the flask became quite gelatinous when cold. Before it was poured into water, therefore, it was again extracted with petroleum spirit (b.p. 40-60° C.) by shaking in a separator. The latter liquid left on evaporation a soft, fatty matter, which was found to be completely soluble in hot alcohol. On treating this solution with a little animal charcoal, and filtering while hot, there separated on cooling a mass of nearly white substance, which, however, when spread on glass and allowed to dry in a vacuum desiccator, became of a dark slate-blue colour. It was completely soluble in chloroform, and the filtered solution left a residue which, after drying *in vacuo* over sulphuric acid and solid paraffin, had a melting point of about 75° C. On analysis it gave the following figures:—

0.1458 gramme of substance gave 0.3816 gramme of CO_2 = 71.4 per cent. C and 0.1466 gramme H_2O = 11.2 per cent. H.

This result was sufficient to prove that the substance in question is not a paraffin nor an alcohol similar, for example, to cholesterol. It was probably a mixture of bodies, and had the general properties of a *wax*.

The dry resin obtained by precipitation with acidulated water is soluble in glacial acetic acid and also in solutions of the alkali hydrates, from which it is again precipitated on the addition of water. When heated with acetic anhydride no crystallisable acetyl derivative could be obtained.

10 grammes of the resin were brought into 50 grammes of solid potassium hydroxide in a state of fusion, and the mixture kept at that temperature for a few minutes. The very dark coloured mass had a phenolic odour, and, when taken up with water and an excess of sulphuric acid added, a black resin was separated, while at the same time an almost intolerable odour was developed, reminding of that of skatol, and which was

possibly due to some derivative of the latter. The liquid was then distilled, and the strongly acid distillate, which had the same unpleasant odour, was neutralised with barium carbonate, but after filtering and evaporating the liquid this odour disappeared. To the concentrated solution of the barium salt a little alcohol was added, when a small amount of a crystalline salt was precipitated. This, when dried at 120-130° C., was analysed with the following result :—0.2652 gramme of the salt gave 0.2364 gramme BaSO_4 = 52.42 per cent. Ba. The acid in question was therefore essentially *acetic acid*, since barium acetate requires 53.7 per cent. Ba. This was also confirmed by the formation of the acetic ester. On the further addition of alcohol to the filtrate from the barium acetate an amorphous salt was obtained which was not suitable for analysis, but which when heated with a little alcohol and sulphuric acid developed the fragrant odour of the esters of the higher fatty acids. The liquid remaining after the distillation of the volatile acids was shaken out with ether, but this afforded no crystallisable or well-defined substance.

THE LEAVES OF ROBINIA PSEUD-ACACIA.

The leaves of the Robinia have been stated to produce poisonous effects when eaten (Blyth, 'Poisons,' 3rd Edit., 1895, p. 465), but, as noted in my previous paper (*Pharm. Rundschau*, New York, 1890, p. 30), it has been recorded in several of the older works that they afford wholesome food for cattle, and may even be used as a substitute for clover. These conflicting statements render it doubtful whether the leaves really possess poisonous properties. A few experiments recently made with them may therefore be mentioned here.

200 grammes of the fresh leaves, collected in the latter part of May, were digested with cold water. The filtered liquid, which contains considerable mucilage, was not coagulated by heat, nor, when acidulated with hydrochloric acid, was it precipitated by any of the alkaloidal reagents. A microscopical examination of the leaves also failed to show the presence of any soluble proteid, such as exists in Robinia bark.

10 grammes of the air-dried leaves, in the form of powder, were digested with Prollius' fluid, the liquid filtered, evaporated, and the residue taken up with acidulated water. This also afforded no indication of the presence of an alkaloid.

Although these chemical tests are of a negative character, in so far as the presence of a soluble proteid or alkaloid is concerned, a more decisive answer to the question would be afforded by some simple physiological experiments, which the writer has been unable to accomplish.

CONCLUSION.

In view of the somewhat extended experimental details of the present investigation, it would seem desirable to briefly summarise some of the observations included therein, and the deductions that may be made from them.

(1) The poisonous proteid—*robin*—the chemical characters of which have now been more fully described, possesses the following general properties. It has an acid reaction, is soluble in water and in salt solutions, and is precipitated from its solution by acids. On heating its aqueous solution it becomes coagulated, although not at a uniform temperature, the largest amount being precipitated between 70 and 80° C. ; at the temperature of a water bath its toxic action is completely destroyed. It affords all the colour reactions of albuminous bodies, and is precipitated by all the commonly-employed reagents. The ash obtained by the ignition of the precipitated proteid contains a considerable amount of iron. All of these reactions appear to be in complete accordance with the accepted characters of a *nucleo-proteid*.

The proteid, when prepared by precipitating a cold concentrated aqueous infusion of the bark with strong alcohol, has furthermore the properties of an enzyme, or contains such a body associated with it. Inasmuch as it is capable of effecting the hydrolysis of both amygdalin and potassium myronate, with the formation respectively of bitter almond oil (*i.e.*, benzaldehyde and hydrocyanic acid) and mustard oil, it appears to resemble the ferment *myrosin*. Like the rennet ferment it is capable of coagulating the casein of milk, and, as has been recently shown by Dr. Carl Lau (*loc. cit.*), the other toxic proteids—ricin, abrin, and crotin—likewise possess this property, as also of agglutinating or clotting the red corpuscles of the blood of certain animals.

(2) The bark contains one or more substances of an alkaloidal nature, which are easily decomposed, even by so weak an alkali as silver hydroxide, with the evolution of ammonia and small amounts of an amine. Although these bodies cannot at present be more definitely defined, owing to the difficulties of their isolation in a pure state, it is very probable that they represent degradation products of the proteid.

No direct evidence has been afforded by the present investigation of the presence of choline in the bark, although the possibility of its presence among the other basic substances is not necessarily excluded.

(3) By the hydrolysis of an extract of the bark with hydro-

chloric acid, a small amount of a crystalline substance has been obtained, agreeing in composition, melting point, and other properties with *syringic acid*, $C_9H_{10}O_5$, together with a red, amorphous substance corresponding to *syringenin*. There is likewise formed by the hydrolysis a dextro-rotatory sugar, the osazone of which has a melting-point agreeing with that of *d*-glucose. These related facts would seem to indicate the presence in the bark of the glucoside *syringin*, $C_{17}H_{24}O_9$. On the other hand, it may be observed that syringic acid is not a product of the direct hydrolysis of syringin, but is formed by the hydrolysis of an intermediate substance resulting from the oxidation of syringin—namely, *gluco-syringic acid*, $C_{15}H_{20}O_{10}$, and there is therefore the possibility that this latter substance may also pre-exist in the bark. Although this can only be determined by the isolation of the initial substances, it may be mentioned that in all the operations for the extraction of the substances from Robinia bark, every precaution was taken to prevent oxidation by evaporating the solutions under diminished pressure.

(4) The bark contains, furthermore, a small amount of tannin, some amorphous colouring matter, a sugar—probably *d*-glucose—and a considerable amount of fatty matter and resin. The resin, as previously shown, is devoid of any marked physiological action, and also possesses no special chemical interest.

In the latter part of this investigation I have been kindly assisted by Dr. H. A. D. Jowett and Mr. F. H. Lees, of the laboratory staff, to whom my thanks are due.

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